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☐ 1. Document ID: US 20030224383 A1

L2: Entry 1 of 16 File: PGPB Dec 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030224383
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DOCUMENT-IDENTIFIER: US 20030224383 A1

TITLE: Atherosclerotic phenotype determinative genes and methods for using the same

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6; 435/287.2, 514/1

ABSTRACT:

Genes whose expression is correlated with and determinant of an atherosclerotic phenotype are provided. Also provided are methods of using the subject atherosclerotic determinant genes in diagnosis and treatment methods, as well as drug screening methods. In addition, reagents and kits thereof that find use in practicing the subject methods are provided. Also provided are methods of determining whether a gene is correlated with a disease phenotype, where correlation is determined using at least one parameter that is not expression level and is preferably determined using a Bayesian analysis.

L2: Entry 1 of 16 File: PGPB Dec 4, 2003

DOCUMENT-IDENTIFIER: US 20030224383 A1
TITLE: Atherosclerotic phenotype determinative genes and methods for using the same

Detail Description Paragraph (97):
[0118] The above gene expression analysis approach to the identification of atherosclerotic phenotype determinative genes may be combined with one or more additional selection protocols in a "multi-prong" gene selection approach for identifying genes associated with an atherosclerotic phenotype. Additional selection protocols that can be employed in conjunction with the subject selection protocol include: (1) selection protocols that identify all currently known genes that are associated with atherosclerosis (e.g., as determined by using existing biological and clinical databases, e.g., by performing a thorough review of the published literature concerning biological research on atherosclerosis mechanism and clinical research related to drugs that have shown a beneficial, or detrimental, effect on patients with atherosclerotic clinical manifestations); (2) genes that have been identified as associated with atherosclerosis using human genetic studies, e.g., genetic linkage analysis (for example, one analyzes the genome of individuals who have presented with premature coronary heart disease (CAD, hard manifestations of CAD before 45 for men and before 50 for women, such as myocardial infarction or

bypass surgery), and their siblings and studies markers within the genome of these individuals that co-segregate with the disease process. The localition of such markers across the entire genome allows for identification of "hot spots" that contain 10-300 genes. These genes become candidates for further analysis); (3) genes that have been identified as associated with atherosclerosis using mouse genetic studies, e.g., using mouse models of human disease (Using established mouse models of atherosclerosis, such as ApoE knock-out mice, one searches for "modifiers" that alter the development of the disease process, either increase or reduce, that come into play upon changing the genetic background of the mice. The modifiers thus identified, or their human equivalents, in turn, become candidate genes for further studies on human atherosclerosis); (4) genes that have been identified as associated with atherosclerosis using epigenetic and methylation studies (It is know that with aging, gene expression can be altered, yet the mechanism(s) for such altered expression remains an enigma. Changes in methylation of CpG islands within the promoter region of a multitude of genes can result in altered transcription of such genes, and we have shown that such changes can occur in cardiovascular tissues with aging. Typically, methylation of the CpG island within the promoter of a gene results in silencing of this gene. Such changes in DNA methylation have been called "epigenetic" as they do not represent necessarily inherited changes. We have been surveying the genome of human aortas for the presence of genes whose methylation is altered within atherosclerotic regions compared to normal aorta tissue. The technique that we have used for this survey is called restriction landmark genome scanning, or RLGS. We have already identified two genes, nucleolin and monocarboxylate transporter 3 (MCT3) that are differently methylated between normal and diseased aorta tissues. These genes have become members of our pool of "candidates"). Where the above expression analysis approach is combined with one or more additional approaches to identify genes that are atherosclerotic phenotype determinative genes, the initial genes identified using each disparate selection protocol may be combined into a single set for further use, as described below, using a number of different combination protocols. For example, each of the initially identified subsets may be additively combined to produce a master set of genes for further use. Alternatively, only the common genes of one or more subsets may be placed in the final set of genes for further use. For example, where one develops five initial subsets of genes using five different selection criteria, such as the specific criteria listed above, only those genes common to at least two or more, three or more, or four or more of the initial subsets, including all of the initial subsets, may be chosen for inclusion in the final set.

☐ 2. Document ID: US 20030215842 A1

L2: Entry 2 of 16 File: PGPB Nov 20, 2003

PGPUB-DOCUMENT-NUMBER: 20030215842
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DOCUMENT-IDENTIFIER: US 20030215842 A1

TITLE: Method for the analysis of cytosine methylation patterns

PUBLICATION-DATE: November 20, 2003

INVENTOR-INFORMATION:

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Sledziewski, Andrew Z.	Shoreline	WA	US	
Schweikhardt, Richard Gary	Seattle	WA	US	

US-CL-CURRENT: 435/6

ABSTRACT:

The present invention provides a novel method for the systematic identification of differentially methylated CpG dinucleotides positions within genomic DNA sequences for use as reliable diagnostic, prognostic and/or staging markers. Particular embodiments comprise genome-wide identification of differentially methylated CpG dinucleotide sequences, further identification of neighboring differentially methylated CpG dinucleotide sequences, and confirmation of the diagnostic utility of selected differentially methylated CpG dinucleotide among a larger set of diseased and normal biological samples. The method, and kits for implementation thereof, are useful in applied assays for the diagnosis, prognosis and/or staging of conditions characterized by differential methylation.

L2: Entry 2 of 16 File: PGPB Nov 20, 2003

DOCUMENT-IDENTIFIER: US 20030215842 A1
TITLE: Method for the analysis of cytosine methylation patterns

Summary of Invention Paragraph (11):
[0009] The applicability of this technique, in many cases, is limited by the few species of enzymes available and the distribution of their corresponding recognition motifs. Furthermore, these techniques are costly, time consuming, and result in the analysis of only individual sites per reaction. Nonetheless, restriction enzyme based technologies have proven utility for genome-wide assessments of methylation patterns, particularly where sequence data is unavailable. Techniques for restriction enzyme based analysis of genomic methylation include the following: differential methylation hybridization (DMH) (Huang et al., Human Mol. Genet. 8, 459-70, 1999); Not I-based differential methylation hybridization (see e.g., WO 02/086163 A1); restriction landmark genomic scanning (RLGS) (Plass et al., Genomics 58:254-62, 1999); methylation sensitive arbitrarily primed PCR (AP-PCR) (Gonzalgo et al., Cancer Res. 57: 594-599, 1997); methylated CpG island amplification (MCA) (Toyota et. al., Cancer Res. 59: 2307-2312, 1999).

Brief Description of Drawings Paragraph (4):
[0027] FIG. 3 shows representative visual output formats of four different art-recognized genome-wide methylation analysis techniques, wherein differential methylation sites are identified by the presence or absence of bands of DNA, or hybridization intensity of spots (DMH). The techniques, from left to right are: Arbitrarily primed-PCR (AP-PCR); Methylated CpG island amplification (MCA); Restriction landmark genomic scanning (RLGS); and Differential methylation hybridization (DMH; also known as ECIST in particular embodiments).

Detail Description Paragraph (55):
[0091] Preferably, the methods used to characterize the methylation patterns of each sample set (hereinafter also referred to as `Discovery techniques`) enable a genome-wide methylation pattern analysis. In a particularly preferred embodiment, the characterization is carried out by means of methylation sensitive restriction enzyme digest analysis, and in particular by means of one or a combination of the following techniques: Methylated CpG island amplification (MCA); Arbitrarily primed PCR (AP-PCR); Restriction landmark genomic scanning (RLGS); Differential methylation hybridization (DMH, also known as ECIST); and NotI restriction based differential hybridization method.

Detail Description Paragraph (60):
[0096] Restriction landmark genomic scanning (RLGS). In RLGS-based methods, differential methylation of CpG positions is discriminated based on digestion of genomic DNA with a methylation sensitive restriction endonuclease. RLGS provides quantitative analysis of CpG islands separated by two-dimensional gel electrophoresis into discrete spots. The resulting spot patterns, or RLGS profiles, are highly reproducible, and thus amenable to intra- and inter-individual comparison.

CLAIMS:

10. The method of claim 9, wherein the methylation-sensitive restriction enzyme based technique is selected from the group consisting of methylated CpG island amplification, arbitrarily-primed polymerase chain reaction, restriction landmark genomic scanning, differential methylation hybridization, Not I restriction-based differential methylation hybridization, and combinations thereof.

☐ 3. Document ID: US 20030211473 A1

L2: Entry 3 of 16 File: PGPB Nov 13, 2003

PGPUB-DOCUMENT-NUMBER: 20030211473
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030211473 A1

TITLE: Cancer diagnostic method based upon DNA methylation differences

PUBLICATION-DATE: November 13, 2003

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6; 435/91.2, 536/24.3

ABSTRACT:

There is disclosed a cancer diagnostic method based upon DNA methylation differences at specific CpG sites. Specifically, the inventive method provides for a bisulfite treatment of DNA, followed by methylation-sensitive single nucleotide primer extension (Ms-SNuPE), for determination of strand-specific methylation status at cytosine residues.

L2: Entry 3 of 16 File: PGPB Nov 13, 2003

DOCUMENT-IDENTIFIER: US 20030211473 A1
TITLE: Cancer diagnostic method based upon DNA methylation differences

Summary of Invention Paragraph (12):
[0011] There is a variety of genome scanning methods that have been used to identify altered methylation sites in cancer cells. For example, one method involves restriction landmark genomic scanning (Kawai et al., Mol. Cell. Biol. 14:7421-7427, 1994), and another example involves methylation-sensitive arbitrarily primed PCR (Gonzalgo et al., Cancer Res. 57:594-599, 1997). Changes in methylation patterns at specific CpG sites have been monitored by digestion of genomic DNA with methylation-sensitive restriction enzymes followed by Southern analysis of the regions of interest (digestion-Southern method). The digestion-Southern method is a straightforward method but it has inherent disadvantages in that it requires a large amount of DNA (at least or greater than 5 .mu.g) and has a limited scope for analysis of CpG sites (as determined by the presence of recognition sites for methylation-sensitive restriction enzymes). Another method for analyzing changes in methylation patterns involves a PCR-based process that involves digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR amplification

(Singer-Sam et al., Nucl. Acids Res. 18:687,1990). However, this method has not been shown effective because of a high degree of false positive signals (methylation present) due to inefficient enzyme digestion of overamplification in a subsequent PCR reaction.

Detail Description Paragraph (18):
[0046] The ability to detect methylation changes associated with oncogenic transformation is of critical importance in understanding how DNA methylation may contribute to tumorigenesis. Regions of DNA that have tumor-specific methylation alterations can be accomplished using a variety of techniques. This will permit rapid methylation analysis of specific CpG sites using the inventive quantitative Ms-SNuPE primer process. For example, techniques such as restriction landmark genomic scanning (RLGS) (Hatada et al., Proc. Natl. Acad. Sci. USA 88:9523-9527, 1995), methylation-sensitive-representational difference analysis (MS-RDA) (Ushijima et al., Proc. Natl. Acad. Sci. USA 94:2284-2289, 1997) and methylation-sensitive arbitrarily primed PCR (AP-PCR) (Gonzalgo et al., Cancer Res. 57: 594-599, 1997) can be used for identifying and characterizing methylation differences between genomes.

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L2: Entry 4 of 16 File: PGPB Sep 25, 2003

PGPUB-DOCUMENT-NUMBER: 20030180779
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030180779 A1

TITLE: Discovery and diagnostic methods using 5-methylcytosine DNA glycosylase

PUBLICATION-DATE: September 25, 2003

INVENTOR-INFORMATION:

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Day, John K.	Renton	WA	US	

US-CL-CURRENT: 435/6; 435/91.2

ABSTRACT:

The present invention provides methods for identification of methylated, and/or potentially methylatable CpG dinucleotides in genomic DNA sequences, and methods for isolating genomic DNA sequences comprising methylated CpG dinucleotide sequences. The present invention further provides methods for comparison of the methylation status of specific CpG dinucleotides, and patterns thereof between normal and diseased genomic DNA sequences, along with methods for determining all potentially methylatable CpG dinucleotides in a genomic DNA sample. Specifically, the present invention discloses a novel use of 5-methylcytosine DNA glycosylase (5-MCDG) in combination with art-recognized DNA base excision repair (BER) enzymes, and in particular embodiments, in combination with DNA methyltransferase to specifically label methylated CpG dinucleotide sequences in genomic DNA sequences.

L2: Entry 4 of 16 File: PGPB Sep 25, 2003

DOCUMENT-IDENTIFIER: US 20030180779 A1
TITLE: Discovery and diagnostic methods using 5-methylcytosine DNA glycosylase

Summary of Invention Paragraph (17):
[0015] Assays for the discovery of novel differentially methylated CpG sequences are less numerous in the art, but include such methods as: restriction landmark genomic scanning ("RLGS"; Eng et al., Nature Genetics 25:101-102, 2000; Costello et al., Nature Genetics 25:132-138, 2000; Zhu et al., Proc. Natl. Acad. Sci. USA 96:8058-8063, 1999); methylated CpG island amplification ("MCA"; Toyota et al., Cancer Res. 59:2307-2312, 1999; WO 00/26401A1), differential methylation hybridization ("DMH"; Yan et al., Clin. Canc. Res. 6:1432-1438, 2000); arbitrarily primed-polymerase chain reaction ("AP-PCR"; Liang et al., Genomics 53:260-268, 1998); and RLGS in combination with virtual genome scans ("VGS"; Rouillard et al., Genome Research 11:1453-1459, 2001) derived from the sequence of the human genome to predict sequence of RLGS fragments (spots).

Summary of Invention Paragraph (18):
[0016] Restriction Landmark Genomic Scanning. For example, restriction landmark genomic scanning ("RLGS") approaches have been employed to identify sequences and regions of differential methylation, and regions so-identified have been cloned and sequenced. RLGS methods take advantage of the fact that specific DNA cleavage by particular restriction enyzmes, such as NotI is methylation sensitive. Moreover, NotI has a CG-rich octanucleotide recognition motif, and cleaves predominantly in CpG-rich "islands." Thus, digestion of genomic DNA with NotI and end-labeling of the NotI staggered ends, followed by further restriction digestion (e.g., with 5-base and/or 6-base recognition sequence enzymes) in combination with 2-dimensional electrophoresis has been used to generate resolved patterns of CpG-island-related fragments having at least one labeled NotI end. Such patterns can be used to compare the methylation status among various genomic DNA samples, and if a particular NotI site is methylated in a test genomic DNA sample, relative to that in normal genomic DNA, no corresponding end labeled fragment(s) will be visible in the RLGS pattern of the test sample (corresponding `spot disappearance,` or absence). Boundary libraries (e.g., of NotI-EcoRV fragments) can then be used to obtain cloned DNA corresponding to such regions.

Summary of Invention Paragraph (19):
[0017] Significantly, however, such prior art RLGS methods for detection of CpG methylation are limited, inter alia, by: (i) the use of only particular methylation-sensitive restriction enzymes, which effectively limits analyses to CpG sequences within CpG island regions; (ii) dependence (for detection) upon NotI end-labeling (or the equivalent); and (iii) upon the disappearance of (more accurately, the absence of) a test DNA spot (i.e., where a particular NotI site in a test DNA sample is methylated and therefore not cleaved by NotI digestion) relative to a corresponding spot present in the normal (test) DNA 2-dimensional pattern. Moreover the current boundary libraries have `holes,` because the EcoRV-EcoRV fragments are excluded.

Summary of Invention Paragraph (22):
[0020] Methylated CpG Island Amplification. Methylated CpG island amplification ("MCA") is a PCR-based technique for rapid enrichment of hypermethylated CG-rich regions, that requires the sequential digestion by a particular methylation sensitive, methylation insensitive isoschizomeric enzyme pair (i.e., SmaI and XmaI, respectively), followed by PCR amplification based on primers that specifically hybridize to adapters ligated to the staggered XmaI ends. Additionally, the restriction sites must be closely situated (<1 kb apart). Thus, as in the case of prior art RLGS applications, the method is primarily limited to particular CpG sequences within CpG-rich genomic regions (Toyota et al., Cancer Res. 59:2307-2312, 1999). Moreover, and the technique is sensitive to artifacts relating to incomplete digestion with SmaI, the methylation sensitive restriction enzyme. The technique can be combined, in a more complex multistep method with substractive hybridization (RDA; representational difference analysis) to obtain cloned fragments enriched for hypermethylated sequences (Id).

Summary of Invention Paragraph (24):
[0022] Thus, methylation-sensitive AP-PCR methods, are limited commensurate with primer choice, and as for RLGS and MCA described above, are primarily biased toward CpG island regions, especially when extensively CG-rich primer sequences are employed (Liang et al., Genomics 53:260-268, 1998). Generally, methylation-sensitive

AP-PCR is subject to many of the same artifacts that limit the effectiveness of MCA methods, such as incomplete digestion by restriction enzymes, and distance between primer sites.

Summary of Invention Paragraph (29):
[0027] Therefore, there is a need in the art for novel methods to identify all novel differentially methylated CpG dinucleotide sequences, where the methods are neither limited to methylation analyses within CpG-rich genomic regions (as is primarily the case for RLGS, AP-PCR, MCA, and DMH applications), nor limited to methylation analyses of CpG dinucleotide sequences within particular restriction enzymes recognition motifs. Additionally, there is a need in the art for methods which provide for positive detection of methylated genomic DNA fragments based on specific labeling of methylated CpG sequences, as opposed to methods based on differential digestion by a methylation-sensitive restriction enzyme followed by indirect or negative detection, based on labeling of restriction enzyme generated ends and identification by virtue of the absence of labeling (as in RLGS methods). Additionally, there is a need in the art to identify those CpG dinucleotide sequences that are potentially methylatable, either at the level of isolated genomic DNA, or at the cellular level in the context of particular cellular physiologies.

Detail Description Paragraph (51):
[0090] Those skilled in the art will also recognize that a variety of restriction enzymes can be employed for such RLGS and VGS embodiments. Particular embodiments use but a single restriction enzyme, whereas others employ a plurality of restriction enzymes, used either individually (e.g., sequentially), or in combination (e.g., sequentially or contemporaneously). Preferably, a plurality of restriction enzymes are used. Generally, restriction endonuclease digestion is useful to appropriately size the genomic DNA fragments to facilitate subsequent analysis and cloning. Preferably, at least one of the restriction enzyme(s) employed recognizes a 6-base or motif cleavage site (e.g., EcoRV; .sup.5'GAT/ATC.sup.3'). Preferably, at least one of the restriction enzyme(s) employed recognizes a 4-base (e.g., MseI, .sup.5'TT/AA.sup.3'), or 5-base (e.g., HinfI; .sup.5'G/ANTC.sup.3') motif cleavage site. Preferably, at least one of the restriction enzyme(s) employed recognizes a 8-base (e.g., NotI; .sup.5'GC/GGCCGC.sup.3') motif cleavage site. Preferably, if only one restriction enzyme is used, it recognizes a 4-base, or 5-base motif cleavage site. Preferably, the restriction enzyme(s) do not disrupt methylated CpG sequences. Preferably, the restriction enzyme(s) do not disrupt any CpG dinucleotide sequences, regardless of methylation status. Preferably, if the restriction enzyme cleavage sites do comprise CpG dinucleotides, they produce staggered ends upon cleavage that can be repaired to retain the integrity of the corresponding genomic CpG methylation status (e.g., NotI; .sup.5'GC/GGCCGC.sup.3').

Detail Description Paragraph (52):
[0091] FIG. 2 illustrates a comparison of the methylation patterns between "Normal" and "Test" genomic DNA samples, according to a preferred RLGS embodiment of the present invention employing a combination of three restriction enzymes (NotI, EcoRV and HinfI) that are routinely employed in prior art RLGS analyses. Here, the test genomic sample (upper-right side of FIG. 2) contains three .sup.MCpG sequences (one within a NotI cleavage site; "*N"), two of which (including that of the NotI cleavage site; "*N") correspond to positions that are not methylated at the corresponding normal genomic DNA positions (upper-left portion of FIG. 2). The normal and test genomic DNA samples are separately digested with EcoRV ("E"; .sup.5'GAT/ATC.sup.3'), treated with 5-methycytosine deglycoslyase, and subjected to base excision repair (BER) in the presence of cytosine-labeled dCTP ("*C") to specifically label methylated CpG sequences present in the respective genomic DNA samples (see upper portion of FIG. 2). Essentially, the methylated cytosine of .sup.MCpG sequences is replaced by labeled cytosine ("*C"; to produce "*CpG") (see middle of FIG. 2).

Detail Description Paragraph (57):
[0096] FIG. 3 illustrates an alternate RLGS embodiment of the present invention that uses the same set of three restriction endonucleases (NotI, EcoRV and HinfI), but (in contrast to the embodiment of FIG. 2) digestion with the methylation-sensitive restriction endonuclease NotI occurs prior to 5-methylcytosine deglycosylase and BER treatments so that methylated NotI sites are not cleaved. Accordingly, genomic DNA

is cleaved with NotI ("N"; .sup.5'GC/GGCCGC.sup.3'), and the resulting staggered ends are repaired (e.g., with T4 DNA polymerase, or SEQUENASE.TM. version 2.0, U.S.B.) using unlabeled deoxynucleotides (in contrast to prior art RLGS procedures, which introduce labeled nucleotides when repairing staggered NotI ends).

Detail Description Paragraph (62):
[0101] One advantage of using the particular combination of NotI, EcoRV and HinfI is that the methylation-sensitive restriction endoclease NotI is known to specifically cleave DNA at CpG islands (i.e, unmethylated positions thereof), and has been routinely employed along with EcoRV and HinfI to differentially compare the methylation status of normal and test genomic DNA samples using RLGS (Eng et al., Nature Genetics 25:101-102, 2000; Costello et al., Nature Genetics 25:132-138, 2000). Thus, the 2-dimensional position/pattern, and in some cases genetic linkage, of many end-labeled (i.e, NotI repaired ends) NotI/EcoRV/HinfI fragments are known in the art, and this information can be used to compliment the present invention by helping to determine the nature (e.g., CpG island or non-CpG island) and linkage of particular spots on the inventive 2-dimensional gels.

Detail Description Paragraph (64):
[0103] Significantly, however, the present invention is not limited by the availability of any particular boundary library. This is because the present inventive methods to not depend on NotI end-labeling, and rather provide for methylation-specific *CpG labeling and detection of, DNA fragments that would not be detectable by prior art RLGS methods (e.g., EcoRV-EcoRV, EcoRV-HinfI, or HinfI-HinfI fragments). Thus, the benefits of virtual genome scanning (VGS) methods can be fully realized, because cytosine-labeled EcoRV-EcoRV, EcoRV-HinfI, or HinfI-HinfI fragments (i.e., positive spots) that would otherwise correspond to `holes` in the prior art NotI/EcoRV boundary libraries can nonetheless be correlated and characterized by virtue of the corresponding virtual DNA fragment sequences.

Detail Description Paragraph (73):
[0112] Such RLGS and/or VGS embodiments are useful for identification of methylated CpG dinucleotide sequences in genomic DNA, or for comparison of the status, extent or pattern of CpG methylation between or among reference and test samples.

Detail Description Paragraph (74):
[0113] Comparison of methylation patterns. Comparisons of methylation patterns between or among normal and diseased tissues, or between a reference pattern and one or more test patterns, are within the scope of the present invention. Such comparisons are made, for example, by gel electrophoresis or RLGS (as illustrated in the embodiments of FIGS. 2 and 3 above). Alternatively, microarrays (described in detail below) are used to determine the status, relative levels, or patterns of methylated vs. unmethylated cytosines at particular genomic positions.

Detail Description Paragraph (75):
[0114] The present invention affords several advantages over prior art RLGS methods for comparison between or among methylation patterns, including but not limited to the following:

Detail Description Paragraph (76):
[0115] First, prior art RLGS detection of CpG methylation is limited by the use of only particular methylation-sensitive restriction enzymes, which effectively limits analyses to a corresponding number of CpG sequences within CG-rich or CpG island regions. By contrast, the present invention allows detection of all methylated and differentially methylated CpG sequences, including those that are neither associated with NotI sites, nor with NotI site-containing CpG islands (NotI cuts predominantly, but not exclusively at CpG islands). Such fragments would go undetected by prior art methods based on fragment labeling by repair of, for example, NotI staggered ends. Any DNA fragment comprising a .sup.MCpG sequence, but unlabeled by virtue of not having a cleaved NotI terminus, for example, would be invisible in prior art methods (e.g., the 2-dimensional spot corresponding to the NotI-containing HinfI-HinfI fragment in the 2-dimensional gel of FIG. 3 could not be analyzed by prior art methods).

Detail Description Paragraph (77):

[0116] Second, prior art RLGS detection of CpG methylation is limited by dependence (for detection) upon end-labeling of DNA fragment termini. By contrast, the present invention specifically labels only genomic fragments that comprise one or more methylated CpG sequences, and not as in the case of prior art methods, all DNA fragments that have a NotI staggered end, and that may or may not comprise methylated CpG sequences unrelated to those present in NotI cleavage sites. Thus, the inventive 2-dimensional gel patterns are not cluttered by labeled spots corresponding to those NotI sites that are unmethylated in both normal and test genomic DNA samples (i.e., largely corresponding to CpG islands with unchanged methylation status). Moreover, prior art artifacts related to artifactual labeling of sheared ends of genomic DNA are also avoided.

Detail Description Paragraph (78):
[0117] Third, prior art RLGS detection of CpG methylation is based upon the disappearance of (more accurately, the absence of) a test DNA spot (i.e., where a particular NotI site in a test DNA sample is methylated and therefore not cleaved by NotI digestion) relative to a corresponding spot present in the normal (test) DNA 2-dimensional pattern. By contrast, the present invention does not depend upon disappearance (absence) of a test DNA spot, but rather on the appearance (presence) of a test DNA spot not present (or present in a reduced intensity) in the corresponding reference pattern from normal genomic DNA. This distinguishing aspect has the advantage of avoiding particular prior art artifacts, such as failing to distinguishing methylation from differential deletion of particular DNA in the respective test genomic sample.

Detail Description Paragraph (90):
[0128] Methylation pattern comparisons. Hypermethylation embodiments employing DNA methytransferase in combination with 5-methylcytosine deglycosylase for comparisons of methylation patterns between or among normal and diseased tissues, or between a reference pattern and one or more test patterns or tissues, are within the scope of the present invention, using amplifiable labels in place of DNA amplification (see below). Such comparisons are made, for example, by gel electrophoresis, or RLGS (VGS) (as illustrated in the embodiments of FIGS. 2 and 3 above). Alternatively, complementary binding (e.g., hybridization)-based assays, such as microarrays (described below) are used to determine the relative levels or patterns of methylated vs. unmethylated cytosine bases at particular genomic positions.

Detail Description Paragraph (93):
[0131] The Biotin- and Fluorescein-labeled genomic DNA fragments are then resolved, for example, using RLGS or microarrays and detected based on the presence of the distinguishable labels as described in detail herein below (e.g., according to a Tyramide Signal Amplification "TSA.TM. protocol involving either sequential exposure to strepavidin-HRP, and anti-Fluorescein-HRP, or independent exposure to strepavidin-HRP, or anti-Fluorescein-HRP), whereby a comparison (either within a genomic DNA sample, or between or among genomic DNA samples) of the existing CpG methylation status, extent, or pattern with the potentially-methylatable CpG methylation status, extent, or pattern is enabled.

CLAIMS:

4. The method of claim 3, wherein resolving, at least in part, the labeled genomic DNA fragments, detecting the resolved fragments based on the presence of the label, and determining the status, extent, or pattern of CpG methylation for one or more CpG sequences of the resolved fragments is accomplished by using a method selected from the group consisting of restriction landmark genomic scanning (RLGS) methods, virtual genome scanning (VGS) methods, and microarray hybridization methods.

37. The method of claim 36, wherein resolving, at least in part, the labeled genomic DNA fragments, detecting the resolved fragments based on the presence of the label, and determining the status, extent, or pattern of CpG methylation for one or more CpG sequences of the resolved fragments is accomplished by using a method selected from the group consisting of restriction landmark genomic scanning (RLGS) methods, virtual genome scanning (VGS) methods, and microarray hybridization methods.

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L2: Entry 5 of 16 File: PGPB Sep 11, 2003

PGPUB-DOCUMENT-NUMBER: 20030170684
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TITLE: Multiplexed methylation detection methods

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fan, Jian-Bing	San Diego	CA	US	

US-CL-CURRENT: 435/6

ABSTRACT:

The present invention is directed to sensitive and accurate multiplexed assays for target analyte detection and detection of methylation in nucleic acid samples.

L2: Entry 5 of 16 File: PGPB Sep 11, 2003

DOCUMENT-IDENTIFIER: US 20030170684 A1
TITLE: Multiplexed methylation detection methods

Summary of Invention Paragraph (18):
[0017] RLGS profiling of methylation pattern of 1184 CpG islands in 98 primary human tumors revealed that the total number of methylated sites is variable between and in some cases within different tumor types, suggesting there may be methylation subtypes within tumors having similar histology (Costello, J. F., Fruhwald, M. C., Smiraglia, D. J., Rush, L. J., Robertson, G. P., Gao, X., Wright, F. A., Feramisco, J. D., Peltomaki, P., Lang, J. C., Schuller, D. E., Yu, L., Bloomfield, C. D., Caligiuri, M. A., Yates, A., Nishikawa, R., Su Huang, H., Petrelli, N. J., Zhang, X., O'Dorisio, M. S., Held, W. A., Cavenee, W. K. and Plass, C. (2000) Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. Nat Genet, 24, 132-138.). Aberrant methylation of a proportion of these genes correlates with loss of gene expression. Based on these observations, in one object of the invention the methylation pattern of a sizable group of tumor suppressor genes or other cancer-related genes will be used to classify and predict different kinds of cancer, or the same type of cancer in different stages.

Detail Description Paragraph (42):
[0074] Other methods such as Restriction landmark genomic scanning (RLGS) (Akama, T. O., Okazaki, Y., Ito, M., Okuizumi, H., Konno, H., Muramatsu, M., Plass, C., Held, W. A. and Hayashizaki, Y. (1997) Restriction landmark genomic scanning (RLGS-M)-based genome-wide scanning of mouse liver tumors for alterations in DNA methylation status. Cancer Res, 57, 3294-3299.; Kawai, J., Hirose, K., Fushiki, S., Hirotsune, S., Ozawa, N., Hara, A., Hayashizaki, Y. and Watanabe, S. (1994) Comparison of DNA methylation patterns among mouse cell lines by restriction landmark genomic scanning. Mol Cell Biol, 14, 7421-7427) and differential methylation hybridization (DMH) (Huang, T. H., Perry, M. R. and Laux, D. E. (1999) Methylation profiling of CpG islands in human breast cancer cells. Hum Mol Genet, 8, 459-470) also find use in the invention. All references are expressly incorporated

herein by reference.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KMC | Draw Desc | Image

☐ 6. Document ID: US 20030099997 A1

L2: Entry 6 of 16 File: PGPB May 29, 2003

PGPUB-DOCUMENT-NUMBER: 20030099997
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030099997 A1

TITLE: Method for gene identification based on differential DNA methylation

PUBLICATION-DATE: May 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bestor, Timothy H.	New York	NY	US	

US-CL-CURRENT: 435/6; 435/91.2

ABSTRACT:

This invention provides a method for detecting the presence of differential methylation between DNA from a first source and the corresponding DNA from a second source. Also provided is a method for determining the presence of a tumor suppressor gene in a DNA sample from a tumor cell.

L2: Entry 6 of 16 File: PGPB May 29, 2003

DOCUMENT-IDENTIFIER: US 20030099997 A1
TITLE: Method for gene identification based on differential DNA methylation

Detail Description Paragraph (45):
[0071] Until recently, the analysis of altered methylation patterns has been limited to a small number of predetermined genes. Traditional molecular biology techniques, such as Southern blotting and polymerase chain reaction (PCR), are not capable of analyzing global methylation patterns and they cannot be used to isolate sequences on the basis of abnormal methylation status. The more recent use of Restriction Landmark Genome Scanning (RLGS) and Methylation-Sensitive Representational Difference Analysis (MS-RDA) have met with limited success. RLGS is a cumbersome, labor-intensive method in which methylation changes are visualized as a dense cluster of "spots" on 2-dimensional gels. This poor resolution presents major difficulties in the identification and isolation of genomic loci, making it unsuitable for high-throughput. MS-RDA is a PCR-based technique that is biased toward short DNA fragments and against GC-rich sequences. Novel array-based methods have also been developed, but these rely heavily on hybridization kinetics. All existing methods are vulnerable to the presence of normal cells in the diseased tissue. With the increasing emphasis on the potential role of methylation in human diseases, there is an immediate need for an effective method for identifying genome-wide changes in DNA methylation in human tissue samples.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KMC | Draw Desc | Image

☐ 7. Document ID: US 20030082556 A1

L2: Entry 7 of 16 File: PGPB May 1, 2003

PGPUB-DOCUMENT-NUMBER: 20030082556
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030082556 A1

TITLE: Binary encoded sequence tags

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kaufman, Joseph C.	Hamden	CT	US	
Roth, Matthew E.	Branford	CT	US	
Lizardi, Paul M.	Wallingford	CT	US	
Feng, Li	Hamden	CT	US	
Latimer, Darin R.	East Haven	CT	US	

US-CL-CURRENT: 435/6; 435/91.2

ABSTRACT:

Disclosed is a method for the comprehensive analysis of nucleic acid samples and a detector composition for use in the method. The method, referred to as Binary Encoded Sequence Tags (BEST), involves generation of a set of nucleic acid fragments; adding an adaptor to the ends containing recognition site for cleavage at a site offset from the recognition site; cleaving the fragment to generate fragments having a plurality sticky ends; indexing of the fragments into sets based on the sequence of sticky ends. The fragments are indexed by adding a offset adaptor to newly generated ends. A different adaptor will be coupled to each different sticky end. The resulting fragments--which will have defined ends, be of equal lengths (in preferred embodiment), and a central sequence derived from the source nucleic acid molecule--are binary sequence tags. The binary sequence tags can be used and further analyzed in numerous ways. For example, the binary sequence tags can be captured by hybridization and coupling, preferably by ligation, to a probe. The probe is preferably immobilized in an array or on sortable beads. One form of the BEST method, referred to as modification assisted analysis of binary sequence tags (MAABST), assesses modification of sequences in nucleic acid molecules by detecting differential cleavage based on the presence or absence of modification in the molecules.

L2: Entry 7 of 16 File: PGPB May 1, 2003

DOCUMENT-IDENTIFIER: US 20030082556 A1
TITLE: Binary encoded sequence tags

Detail Description Paragraph (144):
[0159] MAABST has several advantages over other methods currently used to identify promoters on a genomic scale. Current approaches to identify promoter and other regulatory elements in a high throughput manner include: in silico analysis of nucleotide sequence for transcription factor binding sites, .beta.-lactamase insertion (Whitney et al, A genome-wide functional assay of signal transduction in living mammalian cells, Nat Biotechnol 16(13):1329-33 (1998)). COBRA (Xiong and Laird, COBRA: a sensitive and quantitative DNA methylation assay, Nuc Acid Res 25(12):2532-2534 (1997)), and restriction landmark genomic scanning (Costell et al., aberrant CpG-island methylation has non-random and tumour-type-specific patterns, Nature Genetics 25:132-138 (2000)).

☐ 8. Document ID: US 20020192686 A1

L2: Entry 8 of 16 File: PGPB Dec 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020192686
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020192686 A1

TITLE: Method for epigenetic feature selection

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Adorjan, Peter	Berlin		DE	
Model, Fabian	Berlin		DE	

US-CL-CURRENT: 435/6; 702/19, 702/20

ABSTRACT:

The present invention provides methods and computer program products for epigenetic feature selection. The invention enables the selection of relevant epigenetic features prior to further data analysis. The invention is preferably used for interpretation of large scale DNA methylation analysis data.

L2: Entry 8 of 16 File: PGPB Dec 19, 2002

DOCUMENT-IDENTIFIER: US 20020192686 A1
TITLE: Method for epigenetic feature selection

Summary of Invention Paragraph (7):
[0006] An alternative approach is to look at DNA methylation. 5-methylcytosine is the most frequent covalent base modification in the DNA of eukaryotic cells. It plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis. For example, aberrant DNA methylation within CpG islands is common in human malignancies leading to abrogation or overexpression of a broad spectrum of genes (Jones, P. A., DNA methylation errors and cancer, Cancer Res. 65:2463-2467, 1996). Abnormal methylation has also been shown to occur in CpG rich regulatory elements in intronic and coding parts of genes for certain tumours (Chan, M. F., et al., Relationship between transcription and DNA methylation, Curr. Top. Microbiol. Immunol. 249:75-86,2000). Using restriction landmark genomic scanning, Costello and coworkers were able to show that methylation patterns are tumour-type specific (Costello, J. F. et al., Aberrant CpG-island methylation has non-random and tumor-type-specific patterns, Nature Genetics 24:132-138, 2000). Highly characteristic DNA methylation patterns could also be shown for breast cancer cell lines (Huang, T. H. -M. et al., Hum. Mol. Genet. 8:459-470, 1999).

☐ 9. Document ID: US 20020177154 A1

L2: Entry 9 of 16 File: PGPB Nov 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020177154
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020177154 A1

TITLE: Cancer diagnostic method based upon DNA methylation differences

PUBLICATION-DATE: November 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gonzalgo, Mark L.	South Pasadena	CA	US	
Jones, Peter A.	La Canada	CA	US	
Liang, Gangning	Rowland Heights	CA	US	

US-CL-CURRENT: 435/6; 435/91.2, 536/24.3

ABSTRACT:

There is disclosed a cancer diagnostic method based upon DNA methylation differences at specific CpG sites. Specifically, the inventive method provides for a bisulfite treatment of DNA, followed by methylation-sensitive single nucleotide primer extension (Ms-SNuPE), for determination of strand-specific methylation status at cytosine residues.

DOCUMENT-IDENTIFIER: US 20020177154 A1
TITLE: Cancer diagnostic method based upon DNA methylation differences

Summary of Invention Paragraph (12):
[0011] There is a variety of genome scanning methods that have been used to identify altered methylation sites in cancer cells. For example, one method involves restriction landmark genomic scanning (Kawai et al., Mol. Cell. Biol. 14:7421-7427, 1994), and another example involves methylation-sensitive arbitrarily primed PCR (Gonzalgo et al., Cancer Res. 57:594-599, 1997). Changes in methylation patterns at specific CpG sites have been monitored by digestion of genomic DNA with methylation-sensitive restriction enzymes followed by Southern analysis of the regions of interest (digestion-Southern method). The digestion-Southern method is a straightforward method but it has inherent disadvantages in that it requires a large amount of DNA (at least or greater than 5 .mu.g) and has a limited scope for analysis of CpG sites (as determined by the presence of recognition sites for methylation-sensitive restriction enzymes). Another method for analyzing changes in methylation patterns involves a PCR-based process that involves digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR amplification (Singer-Sam et al., Nucl. Acids Res. 18:687,1990). However, this method has not been shown effective because of a high degree of false positive signals (methylation present) due to inefficient enzyme digestion of overamplification in a subsequent PCR reaction.

Detail Description Paragraph (18):
[0046] The ability to detect methylation changes associated with oncogenic transformation is of critical importance in understanding how DNA methylation may contribute to tumorigenesis. Regions of DNA that have tumor-specific methylation alterations can be accomplished using a variety of techniques. This will permit rapid methylation analysis of specific CpG sites using the inventive quantitative Ms-SNuPE primer process. For example, techniques such as restriction landmark genomic scanning (RLGS) (Hatada et al., Proc. Natl. Acad. Sci. USA 88:9523-9527, 1995), methylation-sensitive-representational difference analysis (MS-RDA) (Ushijima et al., Proc. Natl. Acad. Sci. USA 94:2284-2289, 1997) and methylation-sensitive arbitrarily primed PCR (AP-PCR) (Gonzalgo et al., Cancer Res. 57: 594-599, 1997) can be used for identifying and characterizing methylation differences between genomes.

☐ 10. Document ID: US 20020106649 A1

L2: Entry 10 of 16 File: PGPB Aug 8, 2002

PGPUB-DOCUMENT-NUMBER: 20020106649
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020106649 A1

TITLE: Fixed address analysis of sequence tags

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lizardi, Paul M.	Wallingford	CT	US	
Roth, Matthew E.	Branford	CT	US	
Feng, Li	Hamden	CT	US	
Guerra, Cesar E.	Guilford	CT	US	
Weber, Shane C.	Woodbridge	CT	US	
Kaufman, Joseph C.	Hamden	CT	US	
Latimer, Darin R.	East Haven	CT	US	

US-CL-CURRENT: 435/6; 435/91.2

ABSTRACT:

Disclosed is a method for the comprehensive analysis of nucleic acid samples and a detector composition for use in the method. The method, referred to as Fixed Address Analysis of Sequence Tags (FAAST), involves generation of a set of nucleic acid fragments having a variety of sticky end sequences; indexing of the fragments into sets based on the sequence of sticky ends; associating a detector sequence with the fragments; sequence-based capture of the indexed fragments on a detector array; and detection of the fragment labels. Generation of the multiple sticky end sequences is accomplished by incubating the nucleic acid sample with one or more nucleic acid cleaving reagents. The indexed fragments are captured by hybridization and coupling, preferably by ligation, to a probe. The method allows a complex sample of nucleic acid to be quickly and easily cataloged in a reproducible and sequence-specific manner. One form of the method allows determination of associations, in a nucleic acid molecule, of different combinations of known or potential sequences. Another form of the method assesses modification of sequences in nucleic acid molecules by basing cleavage of the molecules on the presence or absence of modification.

L2: Entry 10 of 16 File: PGPB Aug 8, 2002

DOCUMENT-IDENTIFIER: US 20020106649 A1
TITLE: Fixed address analysis of sequence tags

Detail Description Paragraph (161):

[0176] MAAST has several advantages over other methods currently used to identify promoters on a genomic scale. Current approaches to identify promoter and other regulatory elements in a high throughput manner include: in silico analysis of nucleotide sequence for transcription factor binding sites, .beta.-lactamase insertion (Whitney et al., A genome-wide functional assay of signal transduction in living mammalian cells, Nat Biotechnol 16(13):1329-33 (1998)), COBRA (Xiong and Laird, COBRA: a sensitive and quantitative DNA methylation assay, Nuc Acid Res

25(12):2532-2534 (1997)), and restriction landmark genomic scanning (Costell et al., aberrant CpG-island methylation has non-random and tumour-type-specific patterns, Nature Genetics 25:132-138 (2000)).

☐ 11. Document ID: US 20020086324 A1

L2: Entry 11 of 16 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086324
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020086324 A1

TITLE: Process for high throughput DNA methylation analysis

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Laird, Peter W.	South Pasadena	CA	US	
Eads, Cindy A.	South Pasadena	CA	US	
Danenberg, Kathleen D.	Altadena	CA	US	

US-CL-CURRENT: 435/6; 435/91.2

ABSTRACT:

There is disclosed an improved high-throughput and quantitative process for determining methylation patterns in genomic DNA samples based on amplifying modified nucleic acid, and detecting methylated nucleic acid based on amplification-dependent displacement of specifically annealed hybridization probes. Specifically, the inventive process provides for treating genomic DNA samples with sodium bisulfite to create methylation-dependent sequence differences, followed by detection with fluorescence-based quantitative PCR techniques. The process is particularly well suited for the rapid analysis of a large number of nucleic acid samples, such as those from collections of tumor tissues

L2: Entry 11 of 16 File: PGPB Jul 4, 2002

DOCUMENT-IDENTIFIER: US 20020086324 A1
TITLE: Process for high throughput DNA methylation analysis

Summary of Invention Paragraph (12):
[0010] There are a variety of genome scanning methods that have been used to identify altered methylation sites in cancer cells. For example, one method involves restriction landmark genomic scanning (Kawai et al., Mol. Cell. Biol. 14:7421-7427, 1994), and another example involves methylation-sensitive arbitrarily primed PCR (Gonzalgo et al., Cancer Res. 57:594-599, 1997). Changes in methylation patterns at specific CpG sites have been monitored by digestion of genomic DNA with methylation-sensitive restriction enzymes followed by Southern analysis of the regions of interest (digestion-Southern method). The digestion-Southern method is a straightforward method but it has inherent disadvantages in that it requires a large amount of high molecular weight DNA (at least or greater than 5 .mu.g) and has a limited scope for analysis of CpG sites(as determined by the presence of recognition sites for methylation-sensitive restriction enzymes). Another method for analyzing changes in methylation patterns involves a PCR-based process that involves digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR

amplification (Singer-Sam et al., Nucl. Acids Res. 18:687, 1990). However, this method has not been shown effective because of a high degree of false positive signals (methylation present) due to inefficient enzyme digestion or overamplification in a subsequent PCR reaction.

☐ 12. Document ID: US 20020072059 A1

L2: Entry 12 of 16 File: PGPB Jun 13, 2002

PGPUB-DOCUMENT-NUMBER: 20020072059
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020072059 A1

TITLE: Method of identifying cells using DNA methylation patterns

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Shiota, Kunio	Chiba		JP	
Tanaka, Satoshi	Saitama		JP	
Ohgane, Jun	Tokyo		JP	
Hattori, Naka	Tokyo		JP	

US-CL-CURRENT: 435/6; 702/20

ABSTRACT:

The present invention provides a method of identifying a cell, tissue or nucleus, comprising collecting information on the methylation pattern of DNA isolated from the cell, tissue or nucleus and analyzing the resultant information.

L2: Entry 12 of 16 File: PGPB Jun 13, 2002

DOCUMENT-IDENTIFIER: US 20020072059 A1
TITLE: Method of identifying cells using DNA methylation patterns

Detail Description Paragraph (8):
[0029] Methods for detecting methylation in the obtained DNA are not particularly limited. Any method, e.g., the RLGS technique, MS-PCR technique, Southern blotting, or CpG island microarray technique, may be used to identify methylation patterns. It should be noted that techniques useful in the present invention for obtaining information on methylation are not limited to the above-mentioned techniques. Any technique may be used as long as information on methylation can be obtained with it. Hereinbelow, the method of the invention will be described with reference to the RLGS technique, the MS-PCR technique, Southern blotting, and CpG island microarray technique as non-limiting examples.

Detail Description Paragraph (10):
[0031] The RLGS (restriction landmark genomic scanning) technique is a widely known technique in which recognition sites of restriction enzymes are used as landmarks for detecting methylation. Briefly, DNA is extracted from a test cell or tissue and then digested with a methylation-sensitive restriction enzyme to thereby produce DNA fragments. These fragments are labeled at the 5' end with a labeling material (e.g., .sup.32P) and separated by the first-dimensional electrophoresis. The resultant DNA fragments are digested with restriction enzymes other than the above

methylation-sensitive restriction enzyme and subjected to the second-dimensional electrophoresis. Then, spots are analyzed by autoradiography, etc. Subsequently, a database of spot patterns specific to the test cell or tissue is prepared. By producing such spot patterns for a cell of interest and for cells to be used for comparison and then comparing those spot patterns, the cell of interest is identified. In the present invention, the state of methylation in several thousand regions of genomic DNA can be analyzed at one time by using a methylation-sensitive restriction enzyme.

Detail Description Paragraph (19):
[0040] The analysis of the methylation pattern thus obtained by the RLGS technique may be performed as described below, for example.

Detail Description Paragraph (58):
[0079] FIG. 7 is a flow chart showing an example of cell identification processing according to the identification program of the invention, wherein information on methylation patterns was analyzed by the RLGS technique. As described in Example 1, a spot pattern as shown in FIG. 3 was obtained for one tissue. Of those positions of spots, 167 positions marked with ".smallcircle." were selected (FIG. 3). The presence or absence of a spot at these positions was examined on individual cells or tissues, and the results were expressed schematically as shown in FIG. 5. Hereinbelow, one example of identification processing of cells, etc. will be described with reference to the data shown in FIG. 5.

Detail Description Paragraph (66):
Analysis of Methylation Patterns by the RLGS Technique

Detail Description Paragraph (67):
[0085] In this Example, methylation patterns were analyzed using the RLGS technique as one example.

Detail Description Paragraph (74):
[0092] As a result, about 1,000 RLGS spots were detected (FIG. 3). Of these spots, about 85% were spots constantly detected regardless of types of cells or tissues. Those positions at which the pattern of detected spot differs depending on types of cells or tissues were given identification numbers (Nos. 1-167 in FIG. 3). Examples of some spot patterns detected at those positions are shown in FIG. 4. In FIG. 4, spot #79 is specific to embryonic stem cell and not found in other cells or tissues. Spot #98 is specific to trophoblast stem cell. Spot #91 is specific to placenta or trophoblast cell lineage. Spot #99 is observed in brain and differentiated trophoblast cell. Spot #30 is specific to sperm. On the other hand, spot #27 is not observed in sperm but observed in other cells and tissues. Thus, by giving numbers to those RLGS spots at which difference was observed in methylation patterns, difference was found in 167 spots.

☐ 13. Document ID: US 6383754 B1

L2: Entry 13 of 16 File: USPT May 7, 2002

US-PAT-NO: 6383754
DOCUMENT-IDENTIFIER: US 6383754 B1

TITLE: Binary encoded sequence tags

DATE-ISSUED: May 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kaufman; Joseph C.	Hamden	CT		
Roth; Matthew E.	Branford	CT		
Lizardi; Paul M.	Wallingford	CT		
Feng; Li	Hamden	CT		
Latimer; Darin R.	East Haven	CT		

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2, 436/94

ABSTRACT:

Disclosed is a method for the comprehensive analysis of nucleic acid samples and a detector composition for use in the method. The method, referred to as Binary Encoded Sequence Tags (BEST), involves generation of a set of nucleic acid fragments; adding an adaptor to the ends containing recognition site for cleavage at a site offset from the recognition site; cleaving the fragment to generate fragments having a plurality sticky ends; indexing of the fragments into sets based on the sequence of sticky ends. The fragments are indexed by adding a offset adaptor to newly generated ends. A different adaptor will be coupled to each different sticky end. The resulting fragments--which will have defined ends, be of equal lengths (in preferred embodiment), and a central sequence derived from the source nucleic acid molecule--are binary sequence tags. The binary sequence tags can be used and further analyzed in numerous ways. For example, the binary sequence tags can be captured by hybridization and coupling, preferably by ligation, to a probe. The probe is preferably immobilized in an array or on sortable beads. One form of the BEST method, referred to as modification assisted analysis of binary sequence tags (MAABST), assesses modification of sequences in nucleic acid molecules by detecting differential cleavage based on the presence or absence of modification in the molecules.

131 Claims, 3 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 3

L2: Entry 13 of 16 File: USPT May 7, 2002

DOCUMENT-IDENTIFIER: US 6383754 B1
TITLE: Binary encoded sequence tags

Detailed Description Text (143):
MAABST has several advantages over other methods currently used to identify promoters on a genomic scale. Current approaches to identify promoter and other regulatory elements in a high throughput manner include: in silico analysis of nucleotide sequence for transcription factor binding sites, .beta.-lactamase insertion (Whitney et al., A genome-wide functional assay of signal transduction in living mammalian cells, Nat Biotechnol 16(3):1329-33 (1998)), COBRA (Xiong and Laird, COBRA: a sensitive and quantitative DNA methylation assay, Nuc Acid Res 25(2):2532-2534 (1997)), and restriction landmark genomic scanning (Costell et al., aberrant CpG-island methylation has non-random and tumour-type-specific patterns, Nature Genetics 25:132-138 (2000)).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | PWIC | Draw. Desc | Image

☐ 14. Document ID: US 6331393 B1

L2: Entry 14 of 16 File: USPT Dec 18, 2001

US-PAT-NO: 6331393

DOCUMENT-IDENTIFIER: US 6331393 B1
**** See image for Certificate of Correction ****

TITLE: Process for high-throughput DNA methylation analysis

DATE-ISSUED: December 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laird; Peter W.	South Pasadena	CA		
Eads; Cindy A.	South Pasadena	CA		
Danenberg; Kathleen D.	Altadena	CA		

US-CL-CURRENT: 435/6; 435/91.2

ABSTRACT:

There is disclosed an improved high-throughput and quantitative process for determining methylation patterns in genomic DNA samples based on amplifying modified nucleic acid, and detecting methylated nucleic acid based on amplification-dependent displacement of specifically annealed hybridization probes. Specifically, the inventive process provides for treating genomic DNA samples with sodium bisulfite to create methylation-dependent sequence differences, followed by detection with fluorescence-based quantitative PCR techniques. The process is particularly well suited for the rapid analysis of a large number of nucleic acid samples, such as those from collections of tumor tissues.

26 Claims, 11 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 9

L2: Entry 14 of 16 File: USPT Dec 18, 2001

DOCUMENT-IDENTIFIER: US 6331393 B1
**** See image for Certificate of Correction ****
TITLE: Process for high-throughput DNA methylation analysis

Brief Summary Text (12):
There are a variety of genome scanning methods that have been used to identify altered methylation sites in cancer cells. For example, one method involves restriction landmark genomic scanning (Kawai et al., Mol. Cell. Biol. 14:7421-7427, 1994), and another example involves methylation-sensitive arbitrarily primed PCR (Gonzalgo et al., Cancer Res. 57:594-599, 1997). Changes in methylation patterns at specific CpG sites have been monitored by digestion of genomic DNA with methylation-sensitive restriction enzymes followed by Southern analysis of the regions of interest (digestion-Southern method). The digestion-Southern method is a straightforward method but it has inherent disadvantages in that it requires a large amount of high molecular weight DNA (at least or greater than 5 .mu.g) and has a limited scope for analysis of CpG sites (as determined by the presence of recognition sites for methylation-sensitive restriction enzymes). Another method for analyzing changes in methylation patterns involves a PCR-based process that involves digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR amplification (Singer-Sam et al., Nucl. Acids Res. 18:687, 1990). However, this method has not been shown effective because of a high degree of false positive signals (methylation present) due to inefficient enzyme digestion or overamplification in a subsequent PCR reaction.

☐ 15. Document ID: US 6261782 B1

L2: Entry 15 of 16 File: USPT Jul 17, 2001

US-PAT-NO: 6261782
DOCUMENT-IDENTIFIER: US 6261782 B1

TITLE: Fixed address analysis of sequence tags

DATE-ISSUED: July 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lizardi; Paul M.	Wallingford	CT		
Roth; Matthew E.	Branford	CT		
Feng; Li	Hamden	CT		
Guerra; Cesar E.	Guilford	CT		
Weber; Shane C.	Woodbridge	CT		
Kaufman; Joseph C.	Hamden	CT		
Latimer; Darin R.	East Haven	CT		

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/24.2, 536/24.3

ABSTRACT:

Disclosed is a method for the comprehensive analysis of nucleic acid samples and a detector composition for use in the method. The method, referred to as Fixed Address Analysis of Sequence Tags (FAAST), involves generation of a set of nucleic acid fragments having a variety of sticky end sequences; indexing of the fragments into sets based on the sequence of sticky ends; associating a detector sequence with the fragments; sequence-based capture of the indexed fragments on a detector array; and detection of the fragment labels. Generation of the multiple sticky end sequences is accomplished by incubating the nucleic acid sample with one or more nucleic acid cleaving reagents. The indexed fragments are captured by hybridization and coupling, preferably by ligation, to a probe. The method allows a complex sample of nucleic acid to be quickly and easily cataloged in a reproducible and sequence-specific manner. One form of the method allows determination of associations, in a nucleic acid molecule, of different combinations of known or potential sequences. Another form of the method assesses modification of sequences in nucleic acid molecules by basing cleavage of the molecules on the presence or absence of modification.

154 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

L2: Entry 15 of 16 File: USPT Jul 17, 2001

DOCUMENT-IDENTIFIER: US 6261782 B1
TITLE: Fixed address analysis of sequence tags

Detailed Description Text (185):
MAAST has several advantages over other methods currently used to identify promoters on a genomic scale. Current approaches to identify promoter and other regulatory elements in a high throughput manner include: in silico analysis of nucleotide sequence for transcription factor binding sites, .beta.-lactamase insertion (Whitney et al., A genome-wide functional assay of signal transduction in living mammalian cells, Nat Biotechnol 16(13):1329-33 (1998)), COBRA (Xiong and Laird, COBRA: a sensitive and quantitative DNA methylation assay, Nuc Acid Res 25(12):2532-2534 (1997)), and restriction landmark genomic scanning (Costell et al., aberrant CpG-island methylation has non-random and tumour-type-specific patterns, Nature

Genetics 25:132-138 (2000)).

☐ 16. Document ID: US 6251594 B1

L2: Entry 16 of 16 File: USPT Jun 26, 2001

US-PAT-NO: 6251594
DOCUMENT-IDENTIFIER: US 6251594 B1
**** See image for Certificate of Correction ****

TITLE: Cancer diagnostic method based upon DNA methylation differences

DATE-ISSUED: June 26, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gonzalgo; Mark L.	South Pasadena	CA		
Jones; Peter A.	La Canada	CA		
Liang; Gangning	Rowland Heights	CA		

US-CL-CURRENT: 435/6; 435/91.2, 435/91.21, 536/24.3, 536/24.31, 536/24.32, 536/24.33, 536/25.3

ABSTRACT:

There is disclosed a cancer diagnostic method based upon DNA methylation differences at specific CpG sites. Specifically, the inventive method provides for a bisulfite treatment of DNA, followed by methylation-sensitive single nucleotide primer extension (Ms-SNuPE), for determination of strand-specific methylation status at cytosine residues.

11 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

L2: Entry 16 of 16 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251594 B1
**** See image for Certificate of Correction ****
TITLE: Cancer diagnostic method based upon DNA methylation differences

Brief Summary Text (12):

There is a variety of genome scanning methods that have been used to identify altered methylation sites in cancer cells. For example, one method involves restriction landmark genomic scanning (Kawai et al., Mol. Cell. Biol. 14:7421-7427, 1994), and another example involves methylation-sensitive arbitrarily primed PCR (Gonzalgo et al., Cancer Res. 57:594-599, 1997). Changes in methylation patterns at specific CpG sites have been monitored by digestion of genomic DNA with methylation-sensitive restriction enzymes followed by Southern analysis of the regions of interest (digestion-Southern method). The digestion-Southern method is a straightforward method but it has inherent disadvantages in that it requires a large amount of DNA (at least or greater than 5 .mu.g) and has a limited scope for analysis of CpG sites (as determined by the presence of recognition sites for methylation-sensitive restriction enzymes). Another method for analyzing changes in methylation patterns involves a PCR-based process that involves digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR amplification

(Singer-Sam et al., Nucl. Acids Res. 18:687,1990). However, this method has not been shown effective because of a high degree of false positive signals (methylation present) due to inefficient enzyme digestion of overamplification in a subsequent PCR reaction.

Detailed Description Text (18):
The ability to detect methylation changes associated with oncogenic transformation is of critical importance in understanding how DNA methylation may contribute to tumorigenesis. Regions of DNA that have tumor-specific methylation alterations can be accomplished using a variety of techniques. This will permit rapid methylation analysis of specific CpG sites using the inventive quantitative Ms-SNuPE primer process. For example, techniques such as restriction landmark genomic scanning (RLGS) (Hatada et al., Proc. Natl. Acad. Sci. USA 88:9523-9527, 1995), methylation-sensitive-representational difference analysis (MS-RDA) (Ushijima et al., Proc. Natl. Acad. Sci. USA 94:2284-2289, 1997) and methylation-sensitive arbitrarily primed PCR (AP-PCR) (Gonzalgo et al., Cancer Res. 57: 594-599, 1997) can be used for identifying and characterizing methylation differences between genomes.

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Term	Documents
METHYLATION	18779
METHYLATIONS	482
(1 SAME METHYLATION).USPT,PGPB,JPAB,EPAB,DWPI.	16
(L1 SAME METHYLATION).USPT,PGPB,JPAB,EPAB,DWPI.	16

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